OXFORD

## COMMENTARY

# (R)Evolutionary Therapy: The Potential of Immunotherapy to Fulfill the Promise of Personalized Cancer Treatment

Ravi A. Madan, James L. Gulley

**Affiliation of authors:** Genitourinary Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD.

**Correspondence to:** Ravi A. Madan, MD, Genitourinary Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 10 Center Drive, Building 10, 12N226, Bethesda, MD 20892 (e-mail: madanr@mail.nih.gov).

## Abstract

Since the millennium, personalized medicine has been at the forefront of therapeutic endeavors in medical oncology. The latest technology has given researchers the ability to define cancer at its molecular core. This has led to the development of "targeted therapies," designed to eliminate driver mutations while leaving healthy cells unscathed. Unfortunately, more than 10 years into the targeted molecular therapy era, successes have been infrequent, and toxicity remains largely unchanged compared with relatively indiscriminant, traditional chemotherapy. Emerging data suggests that the malignant clonal heterogeneity within solid tumors is so diverse that targeting one or even several mutations is likely to have minimal, transient impact. In recent years, new therapies have emerged that can effectively stimulate the immune system and improve survival in patients with metastatic disease. Through immune activation, there is the potential to target the cancer with a biologic diversity that can potentially rival the multiplicity of malignant mutations within tumors. Stimulating the immune system to become an evolving adversary against malignant cells may revolutionize cancer therapy in the years to come.

For over a decade, a primary goal of research and development in medical oncology has focused on "personalized medicine." The prevailing motivation was that traditional chemotherapy was too nonspecific in its ability to target the tumor, often resulting in poor response rates and clinically relevant toxicities. Armed with decades of research that helped define cancer at the molecular level, newer pharmacological agents would specifically target cancer cells, leading to selective elimination of malignancies while sparing healthy cells. The harbinger of this new age of medicine was indeed a revolutionary drug, imatinib. Targeting the pivotal BCR-ABL translocation in chronic mylogenous leukemia (CML), the magnitude of the clinical responses was profound and molecular remissions of disease were common ([1]). Overshadowed by the enthusiasm accompanying a new age in therapeutics was that CML was the most targetable of malignancies, with a single initiating mutation in stark contrast to the inherent heterogeneity that is the hallmark of most solid tumors. Undaunted, the field pushed forward to develop molecularly-targeted therapies for most common cancers, even as imatinib resistance in CML began to emerge, suggesting that the most salient and singular driver mutation was evasive ([2]).

The years that followed brought about innumerable agents targeting relevant molecules, both alone and in combination with standard therapies. Successes were limited but substantial, such as erlotinib, sorafenib, and bevacizumab. Despite the initial premise to improve specificity and thereby reduce toxicity, these agents were often associated with side effects akin to those seen with chemotherapy. Furthermore, agents that were thought to be more "promiscuous" (ie, less focused in their targeting) were often favored in development because they impacted multiple molecular pathways. This approach not only broadened the

potential impact of the treatment but also the spectrum of toxicities for the patients.

Within solid tumors, clonal heterogeneity often limited the potential impact of these "targeting agents." Unlike CML, at diagnosis, solid tumors likely cannot trace their oncogenic nature to a single translocation or even one molecular pathway. In recent years, this has become quite clear, as relatively successful agents have seen their clinical benefit curtailed by the multiple mutations inherent in all solid tumors. Vemurafenib targets the critical BRAF mutation in metastatic melanoma, leading to responses in more than half of patients treated and extending survival; however, this agent also highlights the limitations of targeting one mutation (3). Subsequent studies have defined multiple resistance patterns in patients treated with vemurafenib, which lead to treatment failure and recurrent disease (4,5). Furthermore, tumor biopsies from patients treated with vemurafenib have shown multiple mutations within the same biopsy sample, suggesting that the breadth of resistance patterns cannot be overcome with the simple addition of agents that target a secondary oncogenic mutation (5).

The finding of multiple mutations within a biopsy also highlights a potential flaw with biopsy driven treatment selection, an approach that has been investigated in multiple cancers, including lung cancer (6). Given the plethora of targeted agents available, this "precision medicine" strategy involves biopsy of a tumor to determine the driver mutation and then selection of a drug accordingly. This would be akin to determining sensitivities to a bacterial infection, before narrowing the antibiotic therapy. Unfortunately, this strategy does not fully account for the clonal variability found in patients with cancer (7,8). A rapid autopsy study in pancreatic cancer patients highlights the obstacles in biopsy-driven therapy. The investigators sequenced the tumors of seven patients, including both metastatic sites of disease and primary tumors (7). Their findings suggest that not only did the metastatic sites of disease have substantial genetic variability, but the primary tumor itself consisted of numerous genetically disparate malignant subclones that could independently seed secondary sites. Although the investigators hypothesize that these clones all arise from a single parental cell with malignant potential, such a cell would exist years before any diagnosis. If this premise were correct, at the time of biopsy-driven treatment, multiple malignant subclones in the primary tumor would already be in place. The implications here are menacing; biopsies of tumors, whether primary or metastatic, likely give an incomplete genetic picture of the disease at any given time, and thus any treatment based on that biopsy would likely be inadequate with limited durable impact on the broader tumor burden.

Treatment of biologically diverse tumors likely requires more than static, narrowly focused therapies can provide. Even in combination, multiple targeted therapies are likely to be inadequate to deal with the complexity of evolving tumors. Perhaps the infectious disease model can again provide further insight. The immune system itself is inherently designed to deal with the biodiversity of infections, but the concept that immune cells can dispense with malignant cells is only beginning to gain a foothold in clinical oncology. Years of research and the development of relatively crude cytokine-based therapies in the preceding decades has finally given way to an impressive array of immune-based therapies that are emerging from clinical trials with evidence of clinical benefit. Foremost among them are immune checkpoint inhibitors such as ipilumimab, which has demonstrated improved survival in metastatic melanoma (9). Along with agents targeting additional checkpoints such as PD-1 and PDL-1, these therapies aim to limit immune-mediated T-cell

autoregulation, thereby allowing for greater and unfettered antitumor activity. Therapeutic cancer vaccines function differently in that they initially direct immune activation towards tumor-associated antigens with the goal of ultimately generating a broader immune-based, antineoplastic response. Sipuleucel-T is one such vaccine that has improved overall survival in multiple phase III trials in prostate cancer, with additional vaccines in the late stages of clinical development (10).

These immune-based therapies represent different instruments to be used against cancer, and thus a different know-how is required as well. When used as monotherapy, these agents, including sipuleucel-t and ipilumimab, did not induce short-term delays in median progression-free survival in cohorts of advanced metastatic patients, although they did improve survival (9,10). While this was an initial cause for concern about the agents' true efficacy, it may actually highlight the strength of immune-based therapies. Unlike a standard drug treatment, immune activation is likely to persist beyond the period of treatment administration through the activation of immune memory cells. Thus immunotherapy can have a long-term effect on the tumor, perhaps slowing growth over time and thereby improving survival, while immediately appearing like the treatment was unable to delay progression (11). Indeed, some immune data has suggested sustained antitumor beyond the period of therapy (12).

A sustained antitumor immune response is only part of the equation. The greatest impact of immunotherapy is the potential for the activated immune system to evolve to match the multitude of antigens expressed by many cancer clonal populations. This aspect of an induced immune response is known as "antigen spreading" or "antigen cascade." Although immunotherapy may initially direct immune responses to one antigen in particular (vaccines) or nonspecifically (checkpoint inhibitors), that is but the beginning. As cancer cells are killed by immune cells, additional tumor antigens are released in the tumor microenvironment. The activated immune system is capable of processing these additional relevant targets and using them to seek and destroy additional cancer cells bearing those antigens. Although cancer cells are likely to have great genetic biodiversity, they are likely to share many antigens with their sister populations of clones. This overlap in antigen expression, regardless of genetic diversity, will allow the immune response to be enhanced even as the tumor evolves genetically, perhaps developing new oncogenic drivers. As new clonal populations are killed, additional antigens will be released to further broaden diversity of the immune attack, perhaps including multiple aspects of the immune system beyond T-cells as well (13–16). In this manner, the dynamic aspects of the immune system can match the evasive evolutionary capabilities of the tumor, which contribute to resistance patterns seen in response to conventional "targeted therapy."

Multiple clinical trials have already demonstrated evidence of antigen cascade, some associated with improved outcomes (12,16–18). Emerging data suggests the true potential impact of this immune response within the tumor microenvironment. An analysis in patients with colon cancer, a disease where infiltrating T-cells have been associated with improved outcome, demonstrated that the T-cell repertoire found within the tumor had greater diversity than those in adjacent, non–tumor bearing colon tissue (19,20). To further demonstrate the diversity of the response, a similar study in patients with melanoma demonstrated populations of B-cells in addition to T-cells and dendritic cells within the tumor. Analysis of the B-cells demonstrated amplification, clonal diversity, and isotype switching, indicative of affinity maturation against multiple antigens. Together, these data suggest that even in patients not treated with immune

therapies there are varied immune cell populations already present within tumors apparently capable of recognizing the many clonal populations that reside within the microenvironment (21). Activating these populations of immune cells could be the focus of a new generation of cancer therapies.

Despite the therapeutic promise of immunotherapy, there are several potential immune-limiting aspects of the tumor and its microenvironment. Epigenetic defects or mutations in the antigen-processing machinery of tumor cells may prevent the recognition of tumor antigens and enable immune escape. Such characteristics have been associated with higher rates of tumor cell proliferation and poor outcomes (22,23). In addition, regulatory immune cells such as myeloid derived suppressor cells or regulatory T-cells may restrain the immune system from initiating a clinically effective response. Immune-based combinations that capitalize on the immune-modulatory effects of cytotoxic therapies can help overcome some of these obstacles. Radiation and cytotoxic therapies have been shown to enhance antigen presentation, improving immune-mediated tumor cell kill. Some therapies also have the ability to induce an immunogenic form of cell death that can boost an immune response (24,25). Several chemotherapy agents and targeted therapies have also demonstrated the ability to preferentially kill immune-regulating cells (26,27). The favorable immunologic effects of many standard therapies are now becoming more widely understood and appreciated, with ongoing clinical trials looking to capitalize on the possible synergistic immune impact of appropriately selected cytotoxic therapies that can be used to potentiate immunotherapy.

For years medical oncology looked to precise diagnostics and exquisitely targeted therapies to develop the "smart bombs" of anticancer therapy, but perhaps now there is a greater understanding that the true way to personalize anticancer therapy lies within the patients themselves. The inherent biologic masquerade within most tumors likely cannot be overcome by combinations of molecular inhibiting agents, but emerging evidence suggests the immune system is capable of evolving and recognizing the many faces of the cancer within the tumor microenvironment. For the first time we have therapies that are effectively able to incite these insurgent immune cells and improve clinical outcomes. Future studies will investigate what combinations of immune-based therapies can be enhanced with chemotherapy or targeted therapies, and if earlier treatment with immune-based therapies can lead to better outcomes. As medical oncologists know, there is no way to account for the amazing strength and fortitude within cancer patients, but emerging data suggests that each patient's own inherent immunologic dynamism may have the potential to tailor their own therapy against the unique clonal populations within their own tumors. Immunotherapies that effectively activate the latent immunologic warriors can perhaps succeed where past therapies have fallen short, enhancing survival to the point where functional cures may become feasible.

## Note

The authors have no conflicts of interest to report.

## References

1. Kantarjian H, Sawyers C, Hochhaus A, et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med*. 2002;346(9):645–652.
2. Hochhaus A, Kreil S, Corbin AS, et al. Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia*. 2002;16(11):2190–2196.
3. Sosman JA, Kim KB, Schuchter L, et al. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N Engl J Med*. 2012;366(8):707–714.
4. Trunzer K, Pavlick AC, Schuchter L, et al. Pharmacodynamic effects and mechanisms of resistance to vemurafenib in patients with metastatic melanoma. *J Clin Oncol*. 2013;31(14):1767–1774.
5. Van Allen EM, Wagle N, Sucker A, et al. The Genetic Landscape of Clinical Resistance to RAF Inhibition in Metastatic Melanoma. *Cancer Discov*. 2014;4(1):94–109.
6. Kim ES, Herbst RS, Wistuba, II, et al. The BATTLE trial: personalizing therapy for lung cancer. *Cancer Discov*. 2011;1(1):44–53.
7. Yachida S, Jones S, Bozic I, et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature*. 2010;467(7319):1114–1117.
8. Campbell PJ, Yachida S, Mudie LJ, et al. The patterns and dynamics of genomic instability in metastatic pancreatic cancer. *Nature*. 2010;467(7319):1109–1113.
9. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363(8):711–723.
10. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med*. 2010;363(5):411–422.
11. Madan RA, Gulley JL, Fojo T, et al. Therapeutic cancer vaccines in prostate cancer: the paradox of improved survival without changes in time to progression. *Oncologist*. 2010;15(9):969–975.
12. Sheikh NA, Petrylak D, Kantoff PW, et al. Sipuleucel-T immune parameters correlate with survival: an analysis of the randomized phase 3 clinical trials in men with castration-resistant prostate cancer. *Cancer Immunol Immunother*. 2013;62(1):137–147.
13. Lurquin C, Lethe B, De Plaen E, et al. Contrasting frequencies of antitumor and anti-vaccine T cells in metastases of a melanoma patient vaccinated with a MAGE tumor antigen. *J Exp Med*. 2005;201(2):249–257.
14. Carrasco J, Van Pel A, Neyns B, et al. Vaccination of a melanoma patient with mature dendritic cells pulsed with MAGE-3 peptides triggers the activity of nonvaccine antitumor cells. *J Immunol*. 2008;180(5):3585–3593.
15. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity*. 2013;39(1):1–10.
16. Nesslinger NJ, Ng A, Tsang KY, et al. A viral vaccine encoding prostate-specific antigen induces antigen spreading to a common set of self-proteins in prostate cancer patients. *Clin Cancer Res*. 2010;16(15):4046–4056.
17. Gulley JL, Arlen PM, Bastian A, et al. Combining a recombinant cancer vaccine with standard definitive radiotherapy in patients with localized prostate cancer. *Clin Cancer Res*. 2005;11(9):3353–3362.
18. Disis ML, Wallace DR, Gooley TA, et al. Concurrent trastuzumab and HER2/neu-specific vaccination in patients with metastatic breast cancer. *J Clin Oncol*. 2009;27(28):4685–4692.
19. Mlecnik B, Tosolini M, Kirilovsky A, et al. Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. *J Clin Oncol*. 2011;29(6):610–618.
20. Sherwood AM, Emerson RO, Scherer D, et al. Tumor-infiltrating lymphocytes in colorectal tumors display a diversity of T cell receptor sequences that differ from the T cells in adjacent mucosal tissue. *Cancer Immunol Immunother*. 2013;62(9):1453–1461.
21. van Baren N, Coulie PG. Ongoing adaptive immune responses in the microenvironment of melanoma metastases. *Ann N Y Acad Sci*. 2013;1284:62–65.
22. Meissner M, Reichert TE, Kunkel M, et al. Defects in the human leukocyte antigen class I antigen processing machinery in head and neck squamous cell carcinoma: association with clinical outcome. *Clin Cancer Res*. 2005;11(7):2552–2560.

23. Atkins D, Breuckmann A, Schmahl GE, et al. MHC class I antigen processing pathway defects, ras mutations and disease stage in colorectal carcinoma. *Int J Cancer*. 2004;109(2):265–273.

24. Hodge JW, Garnett CT, Farsaci B, et al. Chemotherapy-induced immunogenic modulation of tumor cells enhances killing by cytotoxic T lymphocytes and is distinct from immunogenic cell death. *Int J Cancer*. 2013;133(3):624–636.

25. Gameiro SR, Ardiani A, Kwilas A, et al. Radiation-induced survival responses promote immunogenic modulation to enhance immunotherapy in combinatorial regimens. *Onco-immunology*. 2014;3:e28643.

26. Ghiringhelli F, Menard C, Puig PE, et al. Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. *Cancer Immunol Immunother* 2007;56(5):641–648.

27. Ozao-Choy J, Ma G, Kao J, et al. The novel role of tyrosine kinase inhibitor in the reversal of immune suppression and modulation of tumor microenvironment for immune-based cancer therapies. *Cancer Res.* 2009;69(6):2514–2522.